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Studies upon some Phycomycetes

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STUDIES UPON SOME PHYCOMYCETES

BY

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THESIS

FOR THE

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INTRODUCTION

The purpose of the studies, the results of which this thesis embodies, was to obtain some knowledge of the life-history and local distribution of some of the lower soil and aquatic fungi. Most of the work was done from the systematic point of view and supplemented when feasible by studies of development and life-history. The work, which extended over a period of eight months, was done under the supervision of Dr. J. T. Barrett, to whom I am greatly indebted for his many helpful suggestionsand criticisms.

The material was obtained from soil, water and algae or decaying vegetable substances in water. In all, twenty-six samples of water, algae and vegetable remains were examined. These were collected near Urbana, Illinois; eighty-three soil samples collected in the same vicinity, and forty soil samples from near Harrisburg, Illinois, were also examined. The soil was taken at a distance of from three to six inches below the surface, and usually near the roots of higher plants. An endeavor was made to vary the samples collected with reference to the character and moisture content of the soil. About a double handful of soil was taken for each sample.

The method of examination was as follows: the samples of water, or of algae and water which had been collected were placed in covered glass dishes four inches in diameter and two inches deep. Flies were floated on the surface as 'traps'. The soil samples were placed in the same kind of dishes and enough tap-water was added to leave a free layer of water from one-quarter to one-half inch in depth above the surface of the soil. The substratum, consisting usually of three flies,

was floated on the surface of the tap-water. Sometimes several aphids were used along with the flies. Since the zoospores of the forms sought for are usually more abundant near the surface of the water, care was taken to float the substratum. When allowed to sink it was found to be more badly infested with protozoans and bacteria than when floated. Little trouble was experienced in getting the flies and aphids to float if they were first dried on a piece of filter paper and then gently placed on the water surface. Usually twenty-four hours was sufficient time for the substratum to become infected. At the end of this time the flies or aphids were removed, washed thoroly with distilled water and then placed in Syracuse watch-glasses containing distilled water. In many cases, however, one or two flies were allowed to remain in the large dishes for a longer period in hopes that some forms which might be slower in development would appear on them. In the case of the samples from Harrisburg, Illinois, which were all necessarially collected on the same day, but the examination of which extended over a period of three months with the result that the samples became considerably dried and hardened, the soil was placed in the large dishes and moistened for twenty-four hours before the full amount of water and the substrata were added.

The hyphae of the fungi grew out from the substratum forming a greyish to whitish, more or less dense, woolly fringe about the fly or aphid. The fungi were first examined while in the Syracuse watch-glasses. Further studies were made by transferring bits of the material to slides. Growing cells were used in the study of the discharge and germination of the zoospores, tho where the discharge alone

was to be observed, the plain glass slide without a cover glass was found to be more convenient as the water could easily be changed as often as desired. Where the cultures were contaminated with other organisms or where more of the material was required, the organisms were transferred to other substrata, by placing the fly on which it was growing, or a bit of the fungus dissected out, in a dish of distilled water and then adding the new substratum. The use of the larger dishes gave better results than the small ones in such transfers. The secondary substratum was flies, aphids, or grasshopper eggs. Attempts were also made to grow various forms upon agar and gelatin. These attempts will be discussed later under the species with which they were made.

The Phycomycetes are usually known as the algal-like fungi. They are commonly non-septate, septa in most forms being produced only in the older stages of the plant or where the reproductive bodies are cut off. They are probably primitively aquatic plants but many of them are now terrestial. This fact, together with the fact that some of them are saprophytic while others are parasitic, causes them to show some very interesting variations. The outline which follows, showing the systematic relation of the forms studied, was made by referring to an outline prepared by Dr. Barrett, to Engler and Prantl, and to Rabenhorst. The genera which have members represented in the studies, are underscored.

FUNGI.

I. Class Phycomycetes (Sporangium series).

1. Sub-class Oomycetes.

2. Sub-class Zygomycetes.

II. Class Ascomycetes (Ascus series).

III. Class Basidiomycetes (Basidium series).

I. Class Phycomycetes.

1. Sub-class Oomycetes.

a. Order Chytridiales (Chytridineae).

b. " Ancylistales (Ancylistineae).

c. " Saprolegniales (Saprolegniineae).

d. " Monoblepharidiales (Monoblepharidineae).

e. " Peronosporales (Peronosporineae).

2. Sub-class Zygomycetes.

f. Order Mucorales (Mucorineae).

g. " Entomophthorales (Entomophthorineae).

a. Order Chytridiales (Chytridineae).

1. Family Olpidiaceae.

A. Genus Sphaerita.

B. " Olpidium.

C. " Pseudolpidium.

D. " Olpidiopsis.

E. " Pleotrachelus.

F. " Ectrogella.

G. " Pleolpidium.

2. Family Synchytriaceae.

3. " Rhizidiaceae.

4. " Cladochytriaceae.

5. " Hypochytriaceae.

6. " Oochytriaceae.

c. Order Saprolegniales (Saprolegniineae).

1. Family Saprolegniaceae.

A. Genus Pythiopsis.

B. " Saprolegnia.

C. " Achlya.

D. " Aphanomyces.

E. " Dictyuchus.

F. " Thraustotheca.

G. " Septolegnia.

H. " Aplanes.

2. Family Septomitaceae.

A. Genus Septomitus.

B. " Apodachlya.

C. " Nageliella.

D. " Rhipidium.

E. " Blastocladia.

3. Family Pythiaceae.

A. Genus Nematosporangium.

B. Pythium.

DESCRIPTION OF SPECIES.

Saprolegnia ferax (Gruith) Thuret.

Zoosporangia cylindrically clavate. Oogonia terminal, globular, and borne on main hyphae or stalks which were often quite long. Oogonial walls thick and marked with pits. Antheridia and antheridial branches rarely or never developed. Oospores from six to twenty or thirty - even up to fifty - in an oogonium. Oospores centric, measuring from 19 to 27 μ .

This species appeared upon flies which had been dropped into jars containing Spirogyra. The organism was parasitized and the parasite received much of the attention which should have been given the host.

Altho only terminal oogonia were observed, it is said that they are sometimes intercalar. The oogonial stalks were so long as to be lateral branches rather than stalks. The oogonium usually extended some distance down the stalk before a septum occurred, making it really flask-shaped rather than globular (Fig.1). This neck usually contained from one to three oospores. The large pits which were very conspicuous in the walls of some of the oogonia (Fig.2) were not nearly so prominent in others(Fig.1). The large number of oospores is one of the variable characteristics of the species. While all the oospores measured were within the limits of 19 and 27 μ , Humphrey (10) gives the average diameter as 26 μ . In the single case in which anything resembling antheridial branches was observed, two short straight branches were sent out from the oogonial stalk

just below the basal septum of the oogonium. These bore no antheridia and were in contact with no oogonia. In several cases there was a peculiar structure consisting of a projection sent out from the apical side of the oogonium. This projection, which I have not found mentioned in the literature on Saprolegnia, was usually about twice as long as the diameter of the oogonium and contained from one to four oospores. Since in many of the old cultures the oogonia would break off and drift about in the dishes, the peculiar projection just mentioned may have been caused by an intercalar oogonium breaking loose at its basal septum but remaining attached at the other side, thus giving the appearance of a flask-shaped oogonium with a long free, apically borne neck.

Saprolegnia hypogyna Pringsheim.

Hyphae of medium size, zoosporangia cylindrically globular, or sometimes cylindrical, clavate. Oogonia terminal, with pitted walls. Antheridia produced hypogynally, projecting as the basal wall up into the oogonium. Oospores up to eighteen or twenty in an oogonium. They measured from 22 to 26 μ in diameter, and were usually centric.

This species was found growing upon flies which had been thrown into jars containing algae, chiefly Spirogyra. Oogonia had developed by the time the fungus was observed. These were quite large and in most cases were very conspicuously marked with large pits (Fig.3). Altho most of the oogonia were terminal, several were seen which were cylindrical and not much wider than the hyphae. The spherical oogonia usually had a short basal neck

which contained one or two oospores. The oospores were thick-walled and full of food material, either smooth or granular.

The antheridia, which were the most noticeable characteristic of the species, consisted of a projection from the basal wall of the oogonium and extending up into the oogonial cavity (Fig.3). This projection was usually slightly coiled and divided. Kauffman's (11) experiments showed the great variability of the species with reference to the size and development of the antheridial cell and of the antheridia. Variations of S.hypogyna have been described as varieties of S.ferax, to which it bears a very close relation. The projection, which in most cases seemed to be formed by the growth of the upper side of the basal wall of the oogonium, sometimes had a small cavity or cell below it as tho formed by the spreading to the two sides of the basal wall of the oogonium. The actual contact of antheridia and oospheres was not observed. No signs of any other type of antheridia were seen.

Kauffman (11), in 1908, reported this species for the first time in America. I have found no record of its having been found in this country since that time.

Achlya americana Humphrey.

Hyphae thick. Zoosporangia short and thick, produced abundantly. Oogonia terminal, globular, with pitted walls. They were borne on short, straight, racemosely arranged branches. Antheridial branches abundant, branching, and rising from main hyphae near oogonial stalks. Antheridia cylindric to clavate. Oospores usually

four to seven to an oogonium, excentric, with an average diameter of about 23 μ.

This form, which appeared upon a culture from soil, was the first Achlya to appear on any of the cultures. It agrees very closely with the species A.americana as described by Humphrey (10). One zoosporangium was formed sympodially below the first. Often three would be formed in this way before the first one had discharged (Fig.4). The zoospores escaped thru a common aperture at the tip of the zoosporangium and remained clustered there for some time. This is the typical form of discharge for Achlya. Often the zoospores did not escape from the zoosporangium but germinated at once, sending their germ tubes out thru the zoosporangial wall. That the oogonia had pitted walls was clearly revealed by the use of chloroiodide of zinc, tho it was possible to see these pits under the low power of the microscope without the stain. Often an oogonium would produce an elongated tip which would become enlarged at its terminal end to form a second globular oogonium. Then the contents of the first oogonium would move up and fill the second. The result was a dumb-bell-like affair with the terminal oogonium containing the oospores and the older one remaining empty. It was only in the more mature oospores that the excentric character was noticeable (Fig.6). In these there was usually a large, glistening, excentrically placed oil globule.

The numerous antheridial branches sprang from the same hyphae as the oogonial branches and often quite near them. Very frequently, however, fertilization of an oosphere took place from an antheridium produced on a branch arising from an altogether different

hypha. The antheridial branches were branched and more or less enveloped the oogonium (Fig.5). The antheridia were cylindrically clavate.

Achlya megasperma Humphrey.

Hyphae stout, 1 cm. long. Zoosporangia large and freely developed. Oogonia terminal, globular, with smooth, unpitted walls which were thickened, and borne on short, straight, racemosely arranged stalks. Antheridial branches much branched, arising from main hyphae. Antheridia short-clavate, but not present on all oogonia. Oospores centric, one to eight in an oogonium, and measuring from 30 to 35 μ in diameter, average about 40 μ.

This organism appeared upon flies from a soil culture. On the eighteenth of January a single hypha with several immature zoosporangia, was washed with sterilized water and then transferred to the center of a plate of beef gelatin, which had been very slightly acidified with lactic acid, one drop of acid being used to 15 cc. of the medium. Within twenty-four hours there was a noticeable lateral growth of thin mycelium from the old hypha. Four days after the culture was made, the circular growth was about 2 cm. across, but bacteria were present which softened the gelatin. Another plate was inoculated in exactly the same way, but before the bacteria had spread, bits of the ends of the new mycelium were transferred to another beef gelatin plate. Bacteria appeared here, so another transfer of the tips which seemed most free from contamination was made to a tube slant of beef gelatin, and from this in turn to a second tube and from the second tube to

plates of beef gelatin and potato agar. Bacteria followed here so another transfer of the apparently uncontaminated tips was made from the potato agar plate to a second potato agar plate. The potato agar used was 3% agar and was acidified with lactic acid as described for the beef gelatin. This transfer to the second plate of potato agar - the sixth in the series of transfers - occurred on the sixteenth of February, and was successful. The mycelium grew out radially in all directions. The hyphae were thin and produced lateral branches, some of which grew into the air, curling slightly and giving a woolly appearance to the culture. The growth was very fast at first, but as the agar dried it became slower. It continued growing, however, until it had completely covered the plate which was 9 cm. in diameter. On the sixteenth of April, about 85 days from the time the original bit of mycelium was placed on an artificial medium, a portion of the mycelium and agar were placed in a Syracuse watch-glass with distilled water and some aphids and flies. When first placed in the distilled water the hyphae were crooked, weak, covered with lateral branches, and seemed to be making an effort to get rid of their contents by the formation of zoosporangia (Fig.8). The organism started to grow upon the flies and aphids, but as the hyphae were abnormally short and stout, these flies were put in one of the larger glass dishes which would hold more water. Distilled water and more flies were added. The fungus soon appeared on this second set of flies. It seemed to be growing normally. Some of the hyphae grew to a length of 1 cm. Zoosporangia were produced abundantly, as were oogonia. The thickening of the walls of the oogonia was not entirely regular. The oospores

when young were extremely dark. They had a heavy wall and were filled with oil globules (Fig.7). The average size of the oospores, 40 μ, was a little less than the size, 45 μ, given by Humphrey (10). Many spores in the cultures measured far more than this. The variation in size was probably due to the long cultivation on the artificial media, the organism not having had sufficient time to regain its normal condition. That this long cultivation did affect its growth may be readily seen by a comparison of Figs. 8 and 9 which are drawings of the same structure on the same scale, Fig. 8 being drawn when the organism was first removed from the agar to water and Fig. 9 being drawn from the growth upon the second series of flies. Fig. 10 shows portions of two hyphae which were breaking up to form gemmae. The production of these gemmae, which act as restingcells, was a very characteristic feature of old cultures of both Achlya and Saprolegnia.

The determination of the species was made after the period of cultivation on agar and gelatin. This species, A. megasperma, was the most commmon representative of the genus appearing in my cultures.

Achlya racemosa Hildebrand.

Hyphae stout appearing. Zoosporangia cylindrical. Oogonia borne on short, straight, racemosely arranged branches. Oogonia terminal, globular. Oogonial walls smooth but irregularly thickened on the inside and yellowish brown when old. Antheridial branches short, simple or rarely branched, produced on oogonial stalk, sometimes above and sometimes below the basal wall of oogonium.

Antheridia short, from tips of antheridial branches. Oospores one to seven - usually four - in an oogonium, centric, averaging about 28 μ in diameter.

This very interesting species was obtained from a culture of soil from cultivated ground. The very characteristic antheridia and antheridial branches were the most noticeable features of this species (Fig.11). The branches, which were short and simple - tho one was observed which had divided into three tips (Fig.12) - were produced on the oogonial stalk near the basal septum of the oogonium and either above or below this septum. There were usually two of these branches to each oogonium. They were usually bent until only their tips, which were swollen and cut off to form the short-clavate antheridia, touched the oogonial wall. The brownish coloring of the old oogonial wall is said to be a constant feature. It was quite marked in the specimens studied. The average size of the oospores, 28 μ, is a little larger than that given by Humphrey (10) for the same species, 25 μ.

Aphanomyces (De Bary) species.

Members of this genus appeared in four cultures from soil. Achlya and Pythium were present upon the same flies, but by repeated transfers to fresh flies or aphids the organism was finally obtained practically free from any other fungus. Tho the organism was grown for two months, the oogonia never developed, consequently it was not possible to definitely determine the species.

The hyphae were very thin and slender as compared with the hyphae of Achlya and Saprolegnia. The zoosporangia, which were produced on the tips of the hyphae or of the lateral branches, were long and slender (Fig.13), being about the same width as the hypha or branch. The zoospores escaped thru an apical aperture, but instead of forming an irregular cluster at the tip of the zoosporangium, as in Achlya, they seemed to arrange themselves in a hollow spherical mass (Fig.13). There was a noticeable difference in the width of the zoosporanguim before and after the discharge of the zoospores. The zoospores escaped leaving their empty, hyaline cysts in the clustered mass at the tip of the empty zoosporangium. All the zoospores escaped from their cysts at practically the same time. One case was observed in which thirty-three zoospores escaped within ten minutes after the escape of the first one. In some cases, however, the zoospores never escaped from their cysts but germinated at once (Fig.14). The zoospores, measuring from 6.8 to 8.5 μ, were oval in shape with rather pointed ends (Fig.15). Attached to the side of each, near a large, light colored, vacuolate spot, were two long cilia which were moved with a whip-like motion. This lashing movement of the cilia was not very rapid. The cilia could be distinguished before the spore had made its escape from the cyst.

Unsuccessful attempts were made to grow the fungus on artificial media by streaking the surface of a beef agar plate (acidified) with a platinum loop which had been dipped into a drop of sterilized water containing zoospores.

Thraustotheca clavata (De Bary) Humphrey.

Syn. Dictyuchus clavatus De Bary.

Hyphae stout. Zoosporangia short to long, clavate, produced on tips of sympodially formed branches. Oogonia smooth walled, globular and terminal. Antheridia and antheridial branches not seen. Oospores excentric, usually about six to an oogonium, measuring from 17 to 20 μ across.

This species appeared upon cultures from soil from a garden in Harrisburg, Illinois. So far as it has been possible to ascertain this is the first time this species has been found in America, and the only locality other than near Strassburg, Germany. De Bary (4) found the species in 1880 and cultivated it on flies and mealworms for four years. He named the species Dictyuchus clavatus, but in 1892, Humphrey (10) for reasons to be mentioned later, made it the basis of the new genus Thraustotheca.

The soil from which this species appeared was collected January 1, 1912, but cultures were not made until eighty-five days later, by which time the sample of soil had become quite dry. The hyphae were straight and stout, usually measuring from 40 to 55 μ across. When a zoosporangium was formed on the tip of a hypha, a branch was sent out from below the base of the zoosporangium and on the tip of this branch a second zoosporangium was produced (Fig.16); the branching was repeated until there was a series of zoosporangia, the one nearest the tip being the youngest. The zoosporangia were club-shaped but varied greatly as to length and breadth, some of them being

so short that they were almost spherical while others were so long as to be almost cylindrical. Three of them measured 98 x 170 μ, 65 x 255 μ, and 78 x 173 μ respectively. There were about 125 zoospores in each zoosporangium. While contained in the zoosporangium they were polyhedral in shape because of their mutual pressure. The wall of the sporangium was very fragile and broke down in many places allowing the zoospores to escape. They did this, not by swimming, but rather by slowly tumbling over each other, as tho forced to do so by their own weight, until they spread out in a low, broad irregular mass much as a bag full of potatoes would spread out if the bag were suddenly bursted (Fig.17). Even while the zoosporangial wall was unbroken, the zoospores seemed to be encysted and when they escaped the walls of two adjoining spores were not coalesced but were separate (Fig.18). It was the fragility of the wall of the zoosporangium and the non-coalescence of the spore walls which led Humphrey (10) to found the genus Thraustotheca. In the genus Dictyuchus, in which the species was first placed, the walls of the zoospores coalesce and when the spores escape they leave a network of empty walls behind them.

The zoospores measured from 10 to 12.75 μ in diameter. They were almost spherical after they had recovered from the pressure to which they were subjected while in the zoosporangium. They remained quiet for several hours after the breaking of the zoosporangial wall, - in fact I did not see any while they were in the free, swimming stage. When placed in a hanging drop culture the zoospores usually germinated within 24 hours. Not all of these germinating spores passed thru a swarming stage for some of them germinated while still in

the mass of other spores. Upon germination the zoospores sent out one or two, occasionally three, germ tubes (Fig.19). In the cultures growing on flies, zoospores were produced in such quantities that they covered up the hyphae, making study of the organism quite difficult and the constant growth of fresh cultures necessary.

The oogonia were not produced abundantly in my cultures and those that were observed had already fallen from the stalks and there were no antheridia in contact with them (Fig.20). No antheridial branches were seen. The average number of oospores to an oogonium was about six, tho Fischer (9) says that up to twelve are produced. The measurements of 17 to 20 μ for the diameter of the oospores corresponded pretty closely to Fischer's statement of 17 to 19 μ.

Thraustotheca sinuosa sp. nov.

Hyphae slender, not straight. Zoosporangia long-clavate, from tips of hyphae. Oogonia terminal on twisted stalks of varying length. Antheridia not present. Oospores single, brown, excentric, but loosely filling oogonium when old. Oospores measured from 14 to 24 μ.

This species was found in soil taken from a roadside thicket near Harrisburg, Illinois. It appeared from two samples. The first set of flies floated over the first of these samples did not show any signs of the organism, but the water covering the soil was poured off, and fresh water and substrata were added. In about four days this organism had appeared, forming a dense, white, woolly growth

about the fly, but this growth was not so broad as that formed by T.clavata. The fungus could be transferred very easily to fresh flies and aphids. It was kept growing for over three and one-half months on such substrata. Attempts were made to grow it on artificial media by dissecting out pieces of the mycelium and, after washing them with sterilized water, transferring them to plates of beef agar which had been slightly acidified. Such tranfers uniformly failed to grow.

It was thought when this organism first appeared that it was a Dictyuchus. It resembled very closely Linstedt's (14) Dictyuchus Magnusii until the oogonia were developed. These were smaller than those of D.Magnusii and the antheridia were lacking. The zoosporangia were never formed in a basipetal series as described by Linstedt (14). The non-coalescent character of the zoospore walls also differed from the net-like arrangement of the walls of the zoospores of Linstedt's species.

The hyphae of this species, while they extended straight in any given direction, were twisted or bent in short, rounded zig-zags in a very peculiar and characteristic manner (Figs.21,22). The zoosporangia were produced singly at the tips of the hyphae, tho occasionally they seemed to have been produced by sympodial branching. Each zoosporangium produced an average of about thirty zoospores. These were arranged in two or more irregular rows in the shorter zoosporangia (Fig.27). The longer ones, however, had but a single regularly arranged row at the base for one-third to one-half the length

of the zoosporangium and from there on to the tip there were two or more irregular rows (Fig.28). In the younger zoosporangia there was a thin enclosing wall (Fig.26). The differentiation of the contents of the zoosporangium to form zoospores was observed as follows: At 2.05 P.M. the zoospore plasma masses seemed to be separated by the spore origins. Seven minutes later these masses had become fused again, the contents of the zoosporangium being uniform in appearance. Five minutes later the spore masses had begun to separate again. In fifteen minutes from time of differentiation these polyhedral zoospore masses were quite distinct, the outside sac appearing only as a very thin refractive border. At 3.45 this condition was unchanged. In the older zoosporangia the outer wall seemed to have disappeared yet the zoospores or their empty cysts remained clustered in approximately the same relative positions as when the surrounding wall was present (Fig.29). The fact that the spores stayed together without an enclosing sac seemed to be evidence of some intermediate substance which held them in groups for a time at least. This was the 'Zwischensubstanz' mentioned and studied by De Bary (4) and Buesgen (3) in connection with T.clavata (Dictyuchus clavatus).

The zoospores measured from 10.2 to 13.6 μ in diameter. As mentioned above, they would escape from their cysts, leaving them as separate hyaline circles, never as a network. The zoospores germinated readily in a growing cell after about twenty hours. They sentout one or two slender germ tubes (Fig.30).

The oogonia were produced terminally on slender

branches which were often coiled. Sometimes two oogonia were produced on the same stalk (Fig.23) in which case one would be terminal while the other was terminal on a very short branch given out laterally from the first. The arrangement of the oogonial stalks was racemose. The oogonia were produced so abundantly that their characteristic brown color tinted the whole culture. The general shape of the oogonia was globular, but some of them were slightly and irregularly elongate. The contents of the younger oogonia were masses of very spherical and regular oil globules (Fig.24). The more mature oogonia consisted of a thin brown membrane, often quite irregularly wrinkled and folded, loosely enclosing the spherical oospore (Fig.25). This oospore was spherical with thick brown walls. It usually contained one - sometimes more - large excentrically placed, glistening oil globules and a greyish mass of smaller particles of food material. These oospores averaged about 18 μ in diameter, tho they varied greatly in this respect. No germination of the oospores occurred during the time the cultures were kept going.

Blastocladia strangulata Barrett.

Hyphae regularly branched, stout at base, succeeding branches more slender, pseudo-septate. Zoosporangia globular to elongate, produced on tips, singly and in chains. Usually with several papillae on surface. No sexual stage known. Reddish-yellow, thickwalled resting spores produced much as zoosporangia but never in chains.

Dr. Barrett's paper describing this species is still in the printer's hands so I have not yet examined it. Butler (7) describes a fungus from India which resembles this species very much.

This species was the only representative of the genus studied. It first appeared in soil from the Horticulture Grounds of the University. It later appeared in nine out of the forty samples of soil collected near Harrisburg, Illinois. Its appearance in the two localities studied and at points two hundred miles apart would indicate a probably wide distribution. The plant was easily grown on fresh flies and aphids. It also grew upon a dandelion seed which was inadvertently put into one of the dishes along with some aphids. Three attempts were made to grow the fungus on 1 1/2% beef agar (acidified) plates, by dissecting out bits of mycelium which were bearing zoosporangia, and, after washing them in sterilized water, transferring them to the surface of the agar plates. Two attempts were made on the same medium by allowing the zoosporangia to discharge in sterilized water and streaking some of this water containing the zoospores across the surface of the plate with a platinum loop. None of the efforts to grow the plant on artificial media were successful.

The single plant has a slender, fan-like appearance caused by the spreading of the successive branches. Some of the plants reached a length of 6 mm. There was a large basal cell joined to the substratum by rhizoids and from the tip of this cell arose two, sometimes three or four, branches which in turn produced two or more branches at their tips. There were sometimes as many as six sets of branches in such a series in a single plant. Each of the last set of

branches usually produced a zoosporangium or a resting spore at its tip and from below this a sympodial branch was produced and on its tip a second zoosporangium or resting spore was produced. Sometimes these sympodially produced branches were quite short, giving a clustered appearance to the zoosporangia and resting spores. The mycelium had hyaline walls. The older parts seemed to be empty of any solid matter. In some plants the mycelium was long, slender, and usually straight, but in other specimens it was shorter, broader, and had a more crooked appearance. The pseudo-septa, which were such a noticeable characteristic of the mycelium, were marked by a constricted swelling, i.e. there were two swellings separated by a groove. In many cases, however, there was no swelling, the mycelium being almost smooth and uniform in thickness at the point where the septa were produced. These pseudo-septa (Fig.33) were not uniform in thickness. According to Dr. Barrett, they are probably formed by a circle of processes growing out from the wall of the mycelium toward the center of the mycelial cavity and there becoming fused to form a more or less complete wall or plate.

The production of the zoosporangia on the tips of the branches was described under the discussion of the mycelium. The zoosporangia all had several rounded, hyaline papillae. It was thru one of these that the zoospores escaped (Fig.34). If the bacteria were bad in the culture only a single zoosporangium would be produced at a given place, but in most instances there were at least two (Fig.32) borne one below the other. On the hyphae in one culture which seemed to be especially favorable for the production of zoosporangia, as many as

twelve zoosporangia would be produced in a chain, one being cut off and formed just below the other (Fig.33). The apical zoosporangium in such chains would discharge thru a papilla at its tip while each of the succeeding ones discharged thru a papilla near its upper end.

The zoospores measured from 6.8 to 9.4 μ in diameter. There were about fifty zoospores to a zoosporangium which discharged readily when placed in fresh water. In one zoosporangium which was observed, all but two of the fifty zoospores escaped in 24 minutes, the first thirty escaping in 11 minutes. It took each spore about 15 seconds to squeeze thru the opening after it had once started, and then about 40 seconds to regain its original, apparently spherical, shape. The spores which escaped toward the last took a little longer to go thru the opening but they seemed to regain their shape sooner. After regaining their shape, each spore moved off slowly at first, then going away with a rush. No observations were made of the germination of the zoospores.

The resting spores were produced much in the same manner as the zoosporangia, with the exception that they were never borne in chains. They were egg-shaped with the smaller, basal end flattened (Fig.34). They measured about 32 x 48 μ on an average. When young they were almost black in color, but they turned to a reddish yellow when old, giving a brownish tint to the whole mycelial mass. The wall was quite thick and was covered with what seemed to be small conical pits. The resting spores were full of oil globules. As they matured they broke loose at the base but were still held in

place by athin, hyaline, enclosing sac. Finally this too would give way and the spore would sink to the bottom of the dish. Dishes containing such spores were kept for over two months, the water in some of them being changed frequently; in others being allowed to almost dry up before any more was added. In spite of the varied conditions, the spores in none of the dishes germinated. The germination of the resting spores has been observed by Dr. Barrett, however.

Pythium artotrogus (Montagne) De Bary.

Syn. Artotrogus hyinosporus Montagne.

P.hyinosporum (Mont.) Schroeter.

Hyphae much branched. Zoosporangia or conidia unknown. Oogonia spherical, usually intercalar, with spine-like processes from the oogonial wall, occasionally without spines. Antheridia hypogynal, often one cut off above as well as below the oogonium, tho usually only the single one present. Oospores spherical, smooth, thick-walled, 15 to 23 μ in diameter. Sometimes the oospore was only quite loosely enclosed in the oogonium, but usually it almost completely filled it.

This species appeared in four cultures in which other members of the genus Pythium had been growing. This indicates that it might be a parasite, and on account of its appearance in cultures of other Phycomycetes De Bary and Butler (6) suggest that it is a mycoparasite. Butler (6) states that it can not be known with certainty that this form is a Pythium until its asexual stage is found. It was

probably the oospores of this species which W.Smith described as the oospores of Phytophthora infestans.

The oogonia were produced quite abundantly. They were, for the most part, intercalar (Fig.36) altho an occasional one was produced terminally (Fig.38). The oogonia measured from 18 to 25 μ in diameter. The round-tipped spines varied greatly in size and shape, but measured on the average an additional 4.5 μ. Fig.37 shows an oogonium in which the spines are lacking entirely. The antheridia consisted of a portion of the hypha cut off below the oogonium by a cross wall. Occasionally there was a second antheridial cell cut off above the intercalar oogonium. In many cases the antheridial cell was swollen with the end nearest the oogonium pushed forward as tho trying to reach the oogonium (Fig.37). Not rarely the walls of this antheridial cell were distorted into very irregular spine-like projections.

Pythium sp.

The species upon which the following studies were made, was by far the most common Pythium appearing in my cultures. It is probably P.proliferum De Bary, but unfortunately no attempt was made to accurately determine it.

Observations of the diplanetism of the zoospores were made as follows:

At 2:55 the zoosporangium, or what Atkinson (1) calls the prosporangium was observed in a growing cell. At this time there was a large irregularly shaped vacuole at the base of the prosporangium (Fig.41). The vacuole then seemed to be disappearing and the

protoplasmic contents of the prosporangium became more uniformly distributed.

At 3:29 a small refractive papilla had appeared near the apical end of the sporangium (Fig.43). The vacuoles, of which there were now two, were regular in outline and the plasm was in small, rather regular clumps near the periphery. The vacuoles began to become irregular again (Fig.44) and the protoplasm was losing its regularity as to clumps. The small refractive tip was also pushing out.

At 4.05 this 'germ tube' was about 1/5 the length of the greatest diameter of the prosporangium in length, and the vacuoles and the 'clumps' had disappeared so that the contents appeared to be absolutely uniform (Fig.45). Two minutes later the tip of the 'germ tube' began to expand into a thin walled, hyaline sac, and as fast as the sac was formed, the contents of the prosporangium filled it (Fig.46). The protoplasm of the prosporangium did not break up during discharge, but moved as a single unit, - the first place where it broke contact with the sporangial wall being at the base. In one minute the discharge was complete (Fig.47), the walls of the prosporangium had collapsed slightly, and the basal septum was pushed upward slightly.

Just as soon as the contents had reached the outer sac, the protoplasm began to form small clumps. At 4:15 these clumps, still indefinite in shape (Fig.48) had each broken free from the others and had a slight swaying motion. About 6 minutes later, the clumps of protoplasm or zoospores had taken on a definite reniform shape and were rolling over and against each other rather slowly,

but in another minute this motion became rapid. This rapid motion continued for two minutes before the sac broke and the zoospores quickly escaped, swimming rapidly in every direction. The empty prosporangium, the short hyaline neck or 'germ tube', and a small basal portion of the outer sac were all that was left.

There were about twenty of the zoospores, reniform in shape with the concave side very thin (Fig.49). The cilia could not be distinguished.

The first stage in the diplanetism as interpreted by Atkinson (1), required from the time of the first observation one hour and twelve minutes. This included the development of the 'spore origins' (35 min.), the growth of the 'germ tube' (36 min.), and the escape into the outer sac (1 min.). The secondary stage required about sixteen minutes, consisting of the differentiation of the spores (7 min.) and the swarming period (slow, 7 min. and more rapidly, 2 min.).

Notes upon some Chytridiacean Parasites belonging either to the genus Pseudolpidium or to the genus Olpidiopsis.

Owing to the factthat neither the resting spores nor the sexual stage of this parasite was observed, it could not be determined.

The species was found growing as a parasite on S.ferax which had developed upon flies dropped into jars containing Spirogyra. It was kept growing on this host for about twenty days.

The zoosporangia of the parasite developed in the zoosporangia or near the tips of the hyphae of the Saprolegnia. The infected parts of the host were swollen (Figs.50-54) and could be seen with the naked eye as glistening white bodies.

In the earlier stages of development, the host tissue seemed to radiate out from a central dark portion which was the developing parasite. All the host tissue was soon absorbed and the zoosporangium of the host contained nothingbut the zoosporangia of the parasite. The walls of the host at this stage were often broken or wrinkled (Fig.54), tho they usually retained their approximate shape. From one (Fig.53) to seven (Fig.50) zoosporangia of the parasite were observed to develop in a single zoosporangium of the host. The size as well as the number of the zoosporangia of the parasite varied greatly. They produced a hyaline, thin-walled tube of discharge which usually reached (Fig.52) only to the host wall but occasionally extended clear beyond this wall (Fig.51). The zoospores were seen, but not in motion. No cilia were observed.

In some cases the zoosporangia of the parasite had their walls thicker and very much darker than usual. The contents of such bodies, which may have been resting spores, appeared to be globules of oil and food material. No spines or closely adjacent companion cells were observed.

The species was probably that referred to by Butler (6) as Pseudolpidium (? Olpidiopsis) Saprolegniae (A. Braun) Fischer.

Figure 55 shows three zoosporangia of Pythium sp.

which were attacked by a parasite, probably Pseudolpidium pythii. Few of the zoosporangia of the host were infected.

Explanation of Plates.

All figures were drawn with the aid of a camera lucida. The combinations of objectives and oculars used are as follows: Sp. achro. obj. 16 mm. N.A. 0.25, oc. 8, Figs. 4 and 55; B. & L. achro. obj. 16 mm. N.A. 0.25, oc 10, 88x, Fig. 8 - 10, 16, 17, 21, 31 - 33, 50 - 54; Sp. achro. obj. 4 mm. N. A. 0.85, oc. 8, Figs. 1 - 3, 5, 6, 11, 12, 34; B. & L. achro. obj. 4 mm. N.A. 0.85, oc. 10, 430x, Figs. 7, 13 - 15, 19, 20, 22, 23, 26 - 30, 35, 39 - 49; and B. & L. achro. obj. 1.9 mm. N.A. 1.30, oc. 10, 950x, Figs. 18, 24, 25, 36 - 38.

Plate I.

Figs. 1 - 2. Oogonia of Saprolegnia ferax.

Fig. 3. Oogonium of S. hypogyna.

Fig. 4. Zoosporangia of Achlya americana.

Fig. 5. Oogonium of A. americana, showing antheridial branches.

Fig. 6. Oogonia of A. americana, showing excentric character of oospores.

Plate II.

Fig. 7. Oogonium of A.megasperma.

Fig. 8. Hyphae of A.megasperma producing zoosporangia after removal from agar plate to water.

Fig. 9. Zoosporangia of A.megasperma growing on flies.

Fig. 10. Portions of two hyphae A.megasperma forming gemmae.

Figs. 11 - 12. Oogonia of A.racemosa, showing antheridial branches and antheridia.

Plate III.

Fig. 13. Discharged zoosporangia and zoospore cluster of Aphanomyces sp.

Fig. 14. Zoospores of Aph. sp. germinating while in cluster.

Fig. 15. Zoospores of Aph. sp.

Fig. 16. Hypha of Thraustotheca clavata bearing two zoosporangia.

Fig. 17. Discharging zoosporangium of T. clavata.

Fig. 18. Group of zoospores of T. clavata, showing separate character of walls.

Fig. 19. Germinating zoospores of T. clavata.

Fig. 20. Oogonium of T. clavata.

Plate IV.

Figs. 21 - 22. Hyphae of Thraustotheca sinuosa, showing characteristic twistings or bendings.

Fig. 23. Oogonia of T. sinuosa, showing arrangement of oogonial stalks.

Fig. 24. Immature, irregularly shaped oogonium of T. sinuosa.

Fig. 25. Mature oogonium of T. sinuosa, showing large oil globules.

Figs. 26 - 28. Zoosporangia of T. sinuosa.

Fig. 29. Group of zoospores and zoospore cysts of T. sinuosa.

Fig. 30. Germinating zoospores of T. sinuosa.

Plate V.

Figs. 31 - 32. Blastocladia strangulata, showing method of branching and production of zoosporangia and resting spores.

Fig. 33. Chain of empty zoosporangia of B. strnagulata.

Fig. 34. Zoosporangium and resting spores of B. strangulata.

Fig. 35. Portion of hypha of B. strangulata, showing pseudo-septum.

Figs. 36 - 40. Oogonia of Pythium artotrogus.

Plate VI.

Figs. 41 - 48. Successive stages in formation and discharge of zoospores of Pythium sp.

Fig. 49. Zoospores of Pythium sp.

Fig. 50. Zoosporangia of Chytridiacean parasite in tip of hypha of S. ferax.

Fig. 51 - 53. Same as 50, but showing discharge tubes.

Fig. 54. Chytridiacean parasite, showing the bodies resembling resting spores.

Fig. 55. Three zoosporangia of Pythium sp. attacked by Chytridiacean parasite.

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Plate I

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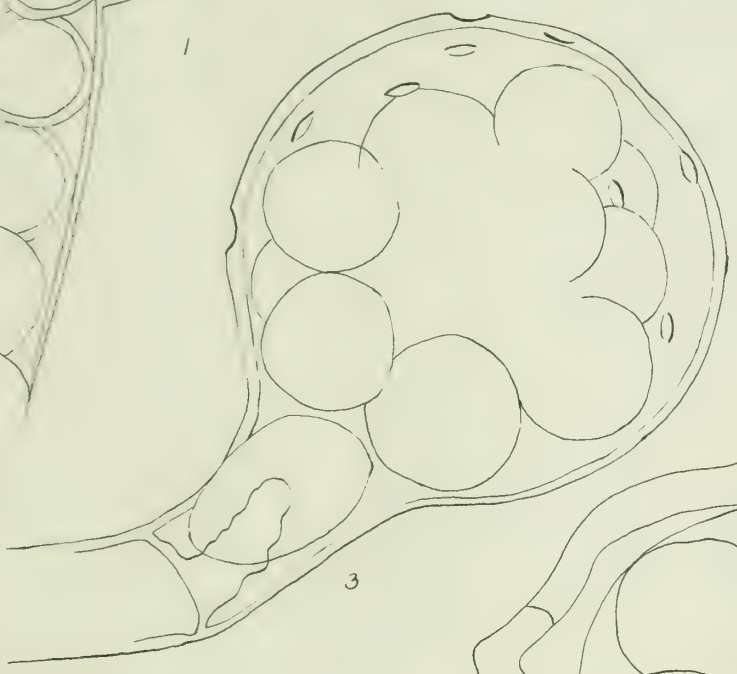

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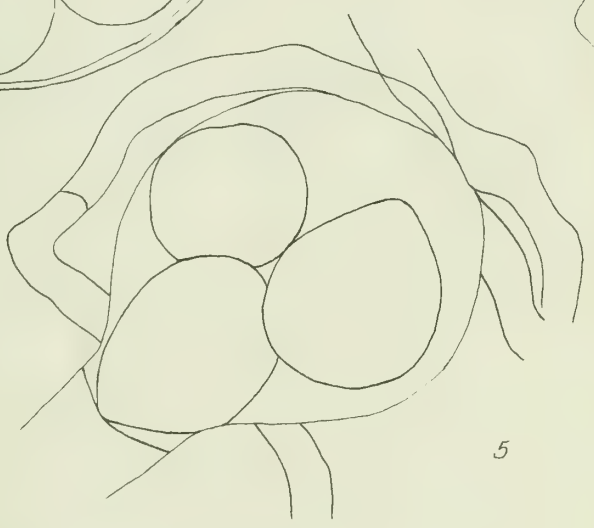

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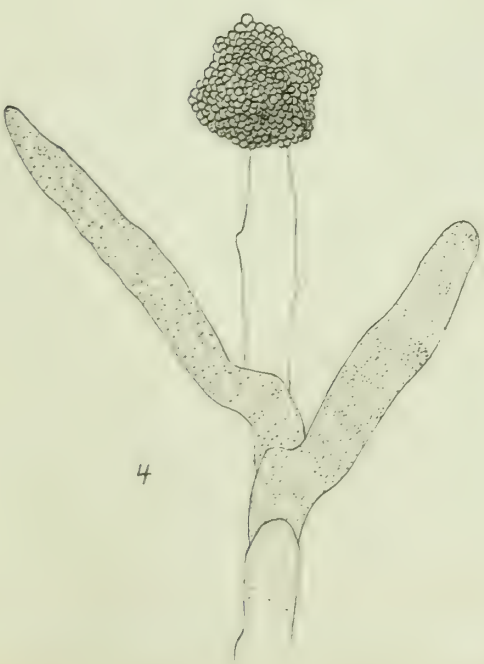

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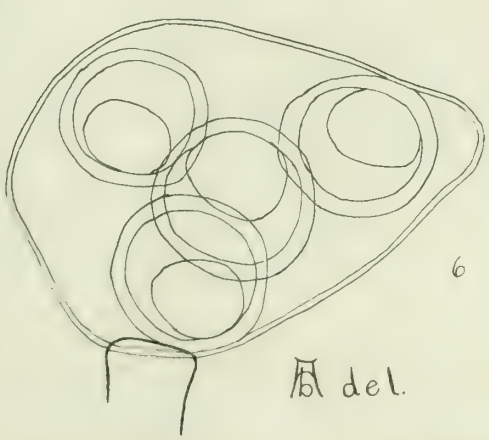

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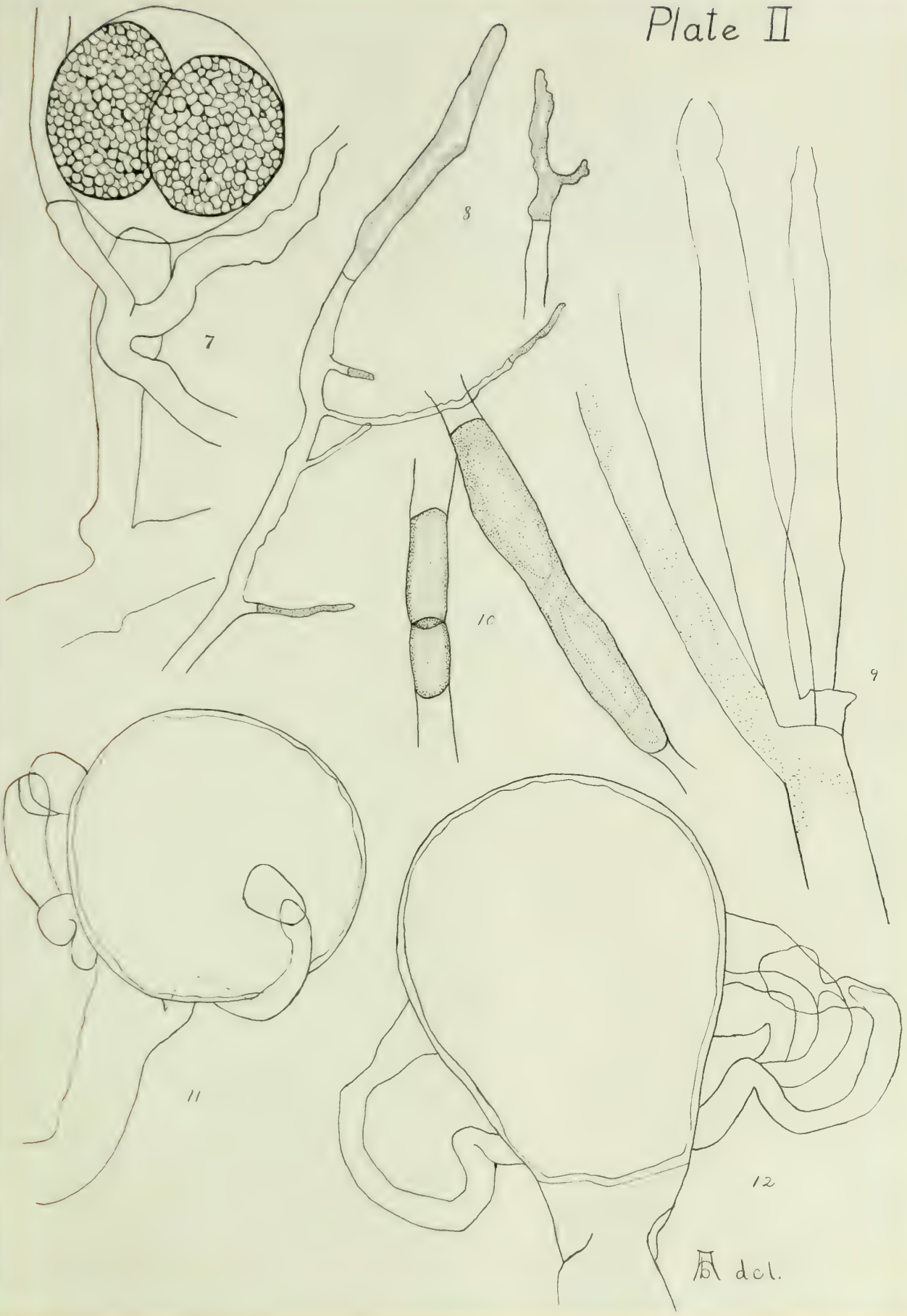

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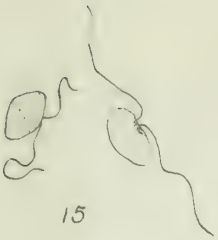

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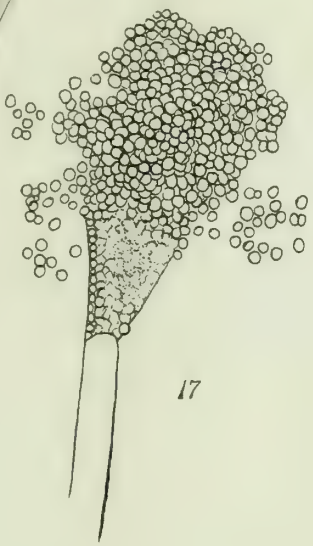

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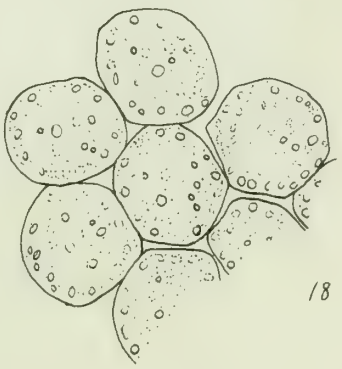

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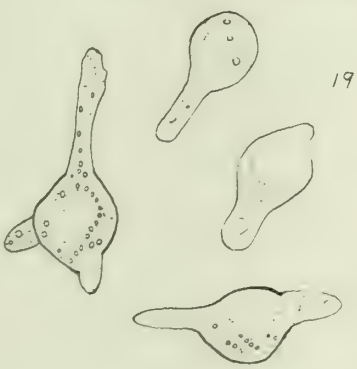

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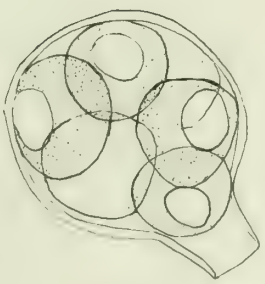

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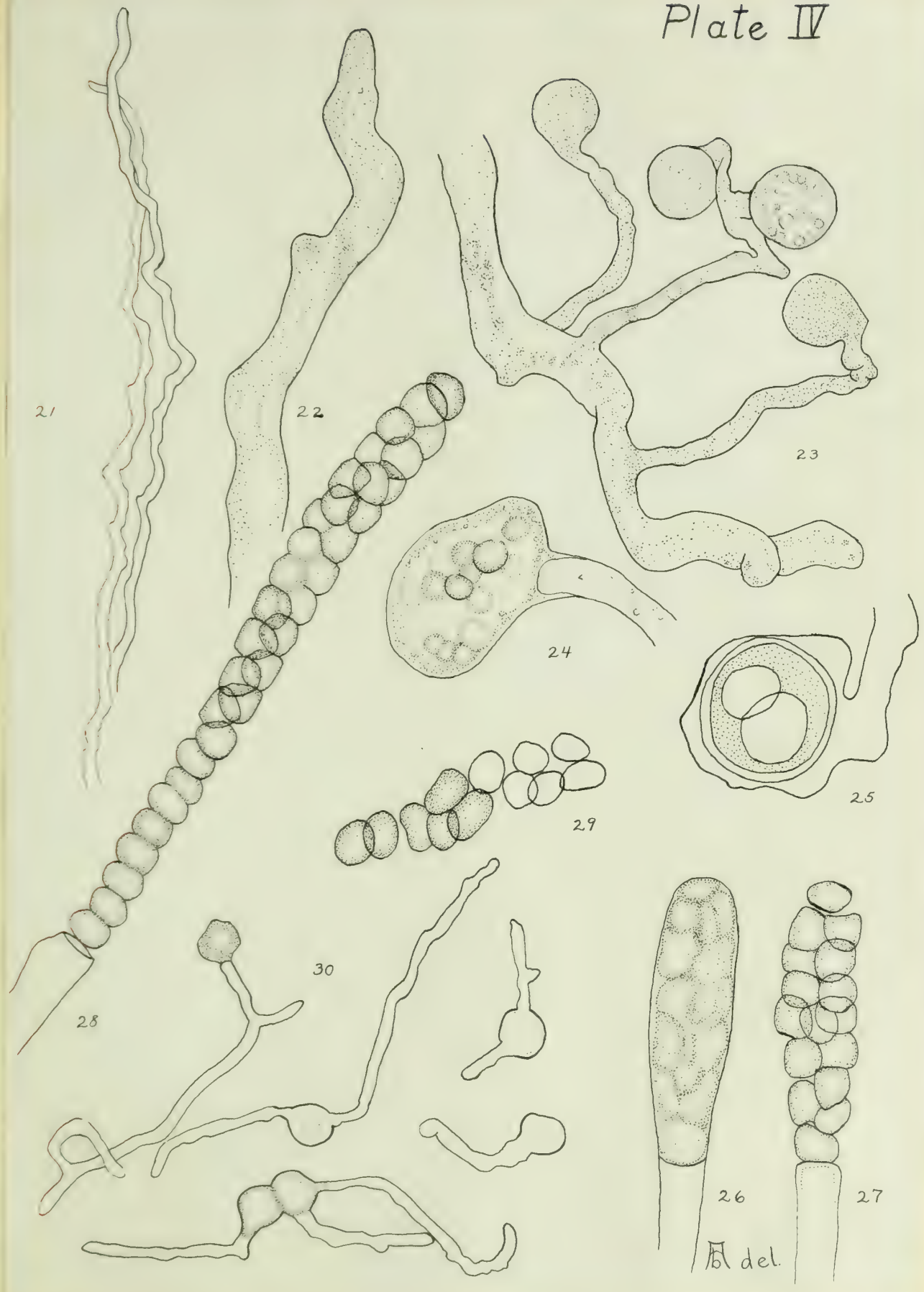
Plate IV



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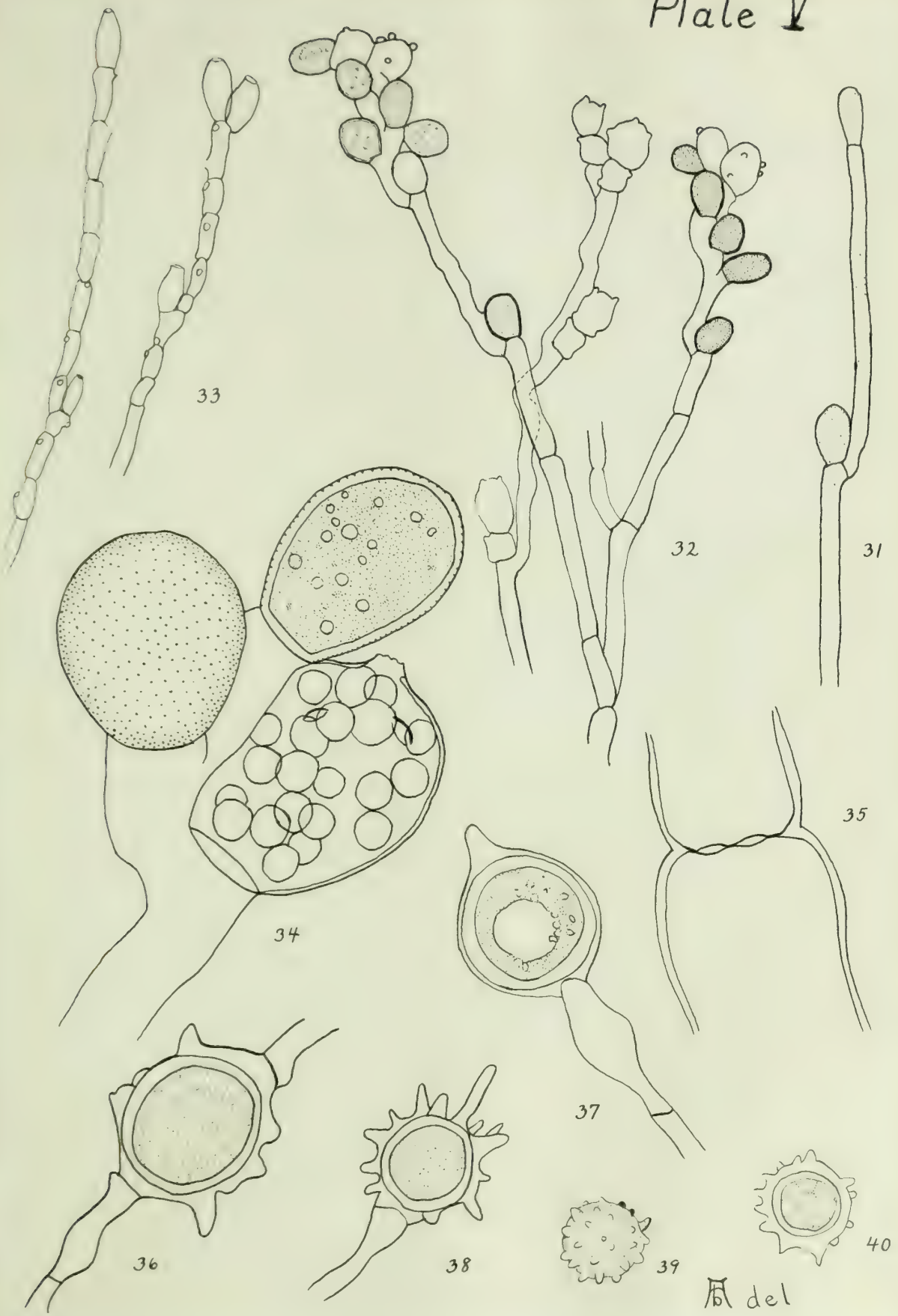

Plate VI

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